

B² triethanolamine/0.25% acetic anhydride. The sections were hybridized overnight, RNase A treated and exposed for 24 days. After development sections were stained with hematoxylin.

IN THE DRAWINGS

Please amend Figure 3 as shown (in red ink) in the attached figure.

REMARKS


Applicants have amended the specification and drawings to comply with the sequence rules (37 CFR §§ 1.821-1.825). Each disclosure of a nucleotide or amino acid sequence in the specification and drawing figures now properly refers to the appropriate sequence identifier. Minor amendments have also been made to correct inadvertent typographical errors.

Applicants also submit a new paper copy of the Sequence Listing and a new identical CRF, together with a statement that the paper copy and CRF are identical. The new Sequence Listing includes SEQ. ID Numbers 11 and 12, which are disclosed in Figure 3 and were inadvertently omitted from the previously submitted Sequence Listing.

No new matter has been entered by the requested amendments. Entry in the file history of the application is requested and favorable consideration of the application is solicited. Attached hereto is a marked-up version of the changes made in the specification by the current amendment. The attached page is entitled "VERSION WITH MARKINGS TO SHOW CHANGES".

The Commissioner is hereby authorized to charge any fee deemed necessary to consider this response timely and/or to enter this paper to Deposit Account No. 19-0365. This paper has been submitted in duplicate.

Respectfully submitted,


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CASE SF0896K

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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In re Application of: :
Alain P. Vicari et al. :
For Patent For: : Group Art Unit: 1614
CHEMOKINES AS ADJUVANTS : Date: June 6, 2002
OF IMMUNE RESPONSE :
Serial No.: 09/768,917 :
Filed: January 24, 2001 :
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Customer Service Center
Initial Patent Examination Division
Assistant Commissioner for Patents
Washington, D.C. 20231

VERSION WITH MARKINGS TO SHOW CHANGES

IN THE SPECIFICATION

The first full paragraph on page 18 has been amended as follows:

RT *RT-PCR analysis*. Semi-quantitative PCR was performed in a Perkin Elmer 9600 thermal cycler, in a final volume of 100 μ l reaction mixture containing 2.5 U AmpliTaq enzyme (5U/ μ l, Perkin Elmer, Paris, France) with its 1X buffer, 0.2 mM of each dNTP (Perkin Elmer, Paris, France), 5% DMSO, and 1 μ M of each forward and reverse primers. CCR6 (Accession No. Z79784) and CCR7 (Accession No. L08176) primers were designed within regions of lowest homology between the chemokine receptors.

+80/CCR6 5'- ATTCAGCGATGTTTTCTGACTC -3' (SEQ ID NO: 1) forward primer,
-1081/CCR6 5'- GGAGAAGCCTGAGGACTTGTA -3' (SEQ ID NO: 2) reverse primer,
+154/CCR7 5'-GATTACATCGGAGACAACACC -3' (SEQ ID NO: 3)

forward primer and

-1202/CCR7 5'-TAGTCCAGGCAGAAGAGTCG -3' (SEQ ID NO: 4) reverse primer were used for RT-PCR and sequencing. For both chemokine receptors, the reaction mixture was subjected to 30 and 35 cycles of PCR with the following conditions : 94°C for 1 min, 61.5°C for 2 min and 72°C for 3 min. PCR products were visualized on 1.2% agarose gels containing 0.5 µg/ml ethidium bromide. Reaction products migrating at the predicted size (1,021 bp for CCR6 and 1,067 bp for CCR7) were gel purified and subcloned into pCRII TA cloning vector (Invitrogen, Leek, The Netherlands) for sequencing verification on an ABI 373A Sequencer (Applied Biosystems, Foster City, CA.) using dye terminator technology. Two other oligonucleotides,

-622/CCR6 5'-GCTGCCTTGGGTGTTGTATTT -3' (SEQ ID NO: 5) and

+662/CCR7 5'-AGAGGAGCAGCAGTGAGCAA -3' (SEQ ID NO: 6), were used as probes for hybridization with the PCR products separated on 1.2% agarose gel and blotted onto Hybond N⁺ membranes (Amersham, Les Ulis, France).

The first full paragraph on page 19 has been amended as follows:

In situ hybridization. *In situ* hybridization was performed as described (Peuchmaur, *et al.*, 1990, *Am. J. Pathol.* 136:383-390). Two couple primers were used for amplifying by RT-PCR the majority of the open reading frame of MIP-3 α (Accession No. D86955) and MIP -3 β [3 α] (Accession No. U77180) genes.

+77/MIP-3 α 5'- TTGCTCCTGGCTGCTTTG -3' (SEQ ID NO: 7) forward primer and

-425/MIP-3 α 5'- ACCCTCCATGATGTGCAAG -3' (SEQ ID NO: 8) reverse primer, +25/MIP-3 β 5'- CTGCTGGTTCTCTGGACTTC -3' (SEQ ID NO: 9) forward primer and

-439/MIP-3 β 5'- CCACTCACACTCACACACAC -3' (SEQ ID NO: 10) reverse primer, were used as described above with an annealing temperature at 62°C. Then, PCR products were cloned into pCRII TA cloning vector (Invitrogen, Leek, The Netherlands) for the generation of sense and anti-sense probes with the adapted promoters. Sense and antisense ³⁵S-labeled probes of MIP-3 α and MIP-3 β , were obtained by run off transcription of the 367 bp and 435 bp fragments, respectively. Six µm

human tonsil sections were fixed in acetone and 4% paraformaldehyde followed by 0.1 M triethanolamine/0.25% acetic anhydride. The sections were hybridized overnight, RNase A treated and exposed for 24 days. After development sections were stained with hematoxylin.